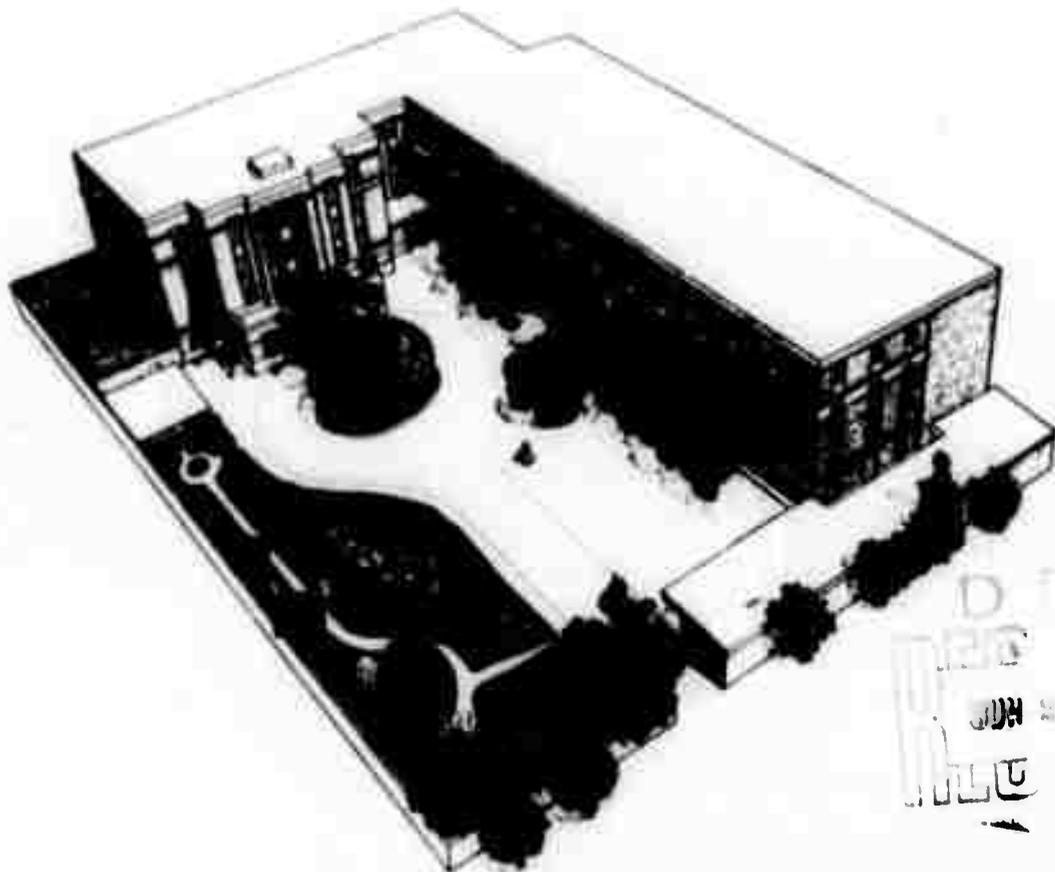




# NAMRU-2

HEMOGLOBIN G HSI-TSOU: 979 Asp-Gly

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## HEMOGLOBIN G HSI-TSOU: $\beta 79$ Asp $\rightarrow$ Gly\*

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### SUMMARY

Hb G Hsi-Tsou was found in Taiwan, in 1964, in two Chinese sisters who were born in Hopei Province. Structure analyses now have shown that the anomaly in this variant is at the  $\beta$ -79 or  $\beta$ EF3 position where a glycyl group replaces the aspartyl group normally present at that location. Another hemoglobin variant found in Chinese subjects, Hb G Taichung, previously has been reported to have the structure change Asp  $\rightarrow$  His at the corresponding location in the  $\alpha$  chain, Position  $\alpha$ EF3 or  $\alpha$ -74.

In the index case separations of the hemoglobin fractions by chromatography with DEAE-Sephadex column revealed that the relative amounts of Hbs  $A_0$  and G Hsi-Tsou were 54 and 46%, respectively. Presence of HbG Hsi-Tsou in the heterozygote apparently causes no anemia.

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### INTRODUCTION

A hemoglobin variant with the singly slow electrophoretic mobility in alkaline pH characteristic of G hemoglobins was found in one female Chinese adult during a population survey<sup>1</sup> in 1964. Recently the variant, named G Hsi-Tsou, was examined by chemical structure studies and found to have a single amino acid substitution in the  $\beta$ T9 peptide. As described below, the aspartyl group normally present at Position  $\beta$ -79 (helical No. EF3) is replaced by a glycyl group; accordingly, Hb G Hsi-Tsou may be represented as  $\alpha_2\beta_2^{79} \text{Asp} \rightarrow \text{Gly}$ .

### MATERIALS AND METHODS

The index subject with Hb G Hsi-Tsou was a 45-year-old Chinese woman who was born in Hopei Province of mainland China. A sister of the index subject

\* The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the U.S. Navy Department or the U.S. Naval Service at large.

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also was found to have the variant. In both cases the subjects were heterozygotes with mixtures of hemoglobins A and G.

The standard methods of chemical structural analysis used in the present study have been described in previous reports<sup>2-7</sup>.

#### RESULTS AND DISCUSSION

The blood hemolysate was examined by starch-gel electrophoresis<sup>8</sup> at pH 8.9 and both Hbs A and G were present. Electrophoresis of the mixture on cellogel<sup>9</sup> showed the G hemoglobin to have an anomalous  $\beta$ -chain. The hemoglobin mixture was separated by column chromatography<sup>10</sup> with DEAE-Sephadex A-50-120 using a Tris-HCl gradient buffer system<sup>7</sup>. The two major components were measured spectrophotometrically in the column effluent and the ratio of Hb A<sub>0</sub> to Hb G was 54 to 46.

Hemoglobin G Hsi-Tsou was subjected to tryptic digestion<sup>2-6</sup> and the peptide mixture examined by mapping<sup>11,12</sup>. The ninhydrin-stained map (Fig. 1) appeared normal except that the spot generally occupied by peptide  $\beta^A T_9$ , Position 1, was missing and a new spot was present at Position 2.

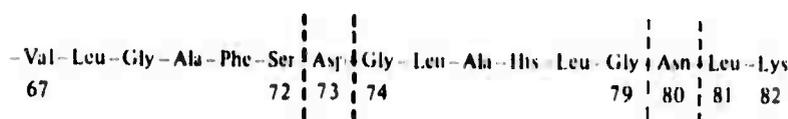
For further analytical work the abnormal peptide,  $\beta^G T_9$ , was separated<sup>7</sup> from the other tryptic peptides by electrophoresis on paper for 2.5 h at 3000 V using pyridine-acetate buffer at pH 5.4, followed by additional electrophoresis at 5000 V for 1.5 h using formate buffer at pH 1.9. For comparative studies normal peptide  $\beta^A T_9$



Fig. 1. Peptide map made from tryptic digest of purified globin from Hb G Hsi-Tsou. Details of electrophoresis and chromatography procedures used for mapping have been described in previous reports<sup>4-6</sup>; 0.2% ninhydrin was used for staining. Peptide  $\beta^A T_9$ , which normally is found at Position 1 on the map, is missing. Parallel to that position chromatographically but displaced to the left electrophoretically is the new spot at Position 2. As described in the text, the new peptide at Position 2 was found to have nearly the same amino acid composition as  $\beta^A T_9$ , except for one more glycyl and one less aspartyl group, and was called  $\beta^G T_9$ . The faint staining pattern of the new peptide, like that normally found for peptide  $\beta^A T_9$ , is due to the occurrence of a valyl residue at the N-terminal position. The increased cathodal mobility of peptide  $\beta^G T_9$  compared with that of  $\beta^A T_9$  results from replacement of one aspartyl group by a glycyl group; the changed mobility matches the slower electrophoretic mobility, in starch gel at alkaline pH, of Hb G Hsi-Tsou compared with that of Hb A<sub>0</sub>.



partyl residues<sup>12</sup>. As shown in Fig. 2, five peptides should be produced by this treatment of peptide  $\beta^A T_9$ . These include two larger peptides: the neutral hexapeptide from Position  $\beta$ -67 to  $\beta$ -72 and the basic pentapeptide from  $\beta$ -74 to  $\beta$ -78. All five of the peptides were found. In addition, free aspartic acid was identified; it was released from Positions  $\beta$ -73 and  $\beta$ -79 by the selective hydrolysis<sup>12</sup> of the  $\beta^A T_9$  peptide under the relatively mild hydrolytic conditions provided at 110° by 0.03 M HCl. All five peptides from  $\beta^A T_9$  had the expected amino acid compositions. No sequence studies were made on the five peptides but amounts of individual amino acids were determined in selected peptides<sup>14</sup>. For example, the basic pentapeptide contained one glycine, two leucines, one alanine and one histidine. From these data and the known sequence of peptide  $\beta^A T_9$  it was assumed that the pentapeptide had the structure Gly-Leu-Ala-His-Leu. Significantly, as described below, this peptide was not found in  $\beta^G T_9$  except as part of a larger peptide.



#### Peptide $\beta^G T_9$

Fig. 3. Peptide  $\beta^G T_9$ , like  $\beta^A T_9$ , contains 16 amino acid residues from valyl at Position  $\beta$ -67 to lysyl at  $\beta$ -82. From experimental results described in the text, Gly replaces Asp at Position  $\beta$ -79. The vertical dashed lines indicate the peptide bonds which would be expected to undergo specific cleavage from hydrolysis with 0.03 M HCl hydrolysis<sup>12</sup>.

The same study of peptides released by selective cleavage of aspartyl bonds in peptide  $\beta^G T_9$  produced differences (Fig. 3). Four peptides were found on the peptide map instead of the five for peptide  $\beta^A T_9$ . One neutral hexapeptide had the same map location and the same amino acid composition as the neutral hexapeptide from peptide  $\beta^A T_9$  and was judged to be Val-Leu-Gly-Ala-Phe-Ser from positions  $\beta$ -67 to  $\beta$ -72. Its presence indicated selective cleavage between Positions  $\beta$ -72 and  $\beta$ -73 and thereby showed that the aspartyl group must still be at Position  $\beta$ -73 in  $\beta^G T_9$  peptide as it is in  $\beta^A T_9$  peptide. The basic pentapeptide Gly-Leu-Ala-His-Leu described above as present in  $\beta^A T_9$  was not present in the  $\beta^G T_9$ ; in its place was a basic peptide containing two glycine and two leucine groups and one each of alanine and histidine. This was assumed to be the hexapeptide Gly-Leu-Ala-His-Leu-Gly which would represent Positions  $\beta$ -74 to  $\beta$ -79 in  $\beta^G T_9$  including the new glycyl residue at position  $\beta$ -79 replacing the aspartyl group found at that position in  $\beta^A T_9$ . In addition there was a basic peptide containing glycine, leucine, alanine, histidine, asparagine (aspartic acid after hydrolysis with 5.7 M HCl prior to amino acid analysis) and lysine. This was assumed to be the larger peptide, illustrated in Fig. 3, extending from glycine at Position  $\beta$ -74 to C-terminal lysine at  $\beta$ -82. The basic dipeptide leucyllysine, representing Positions  $\beta$ -81 and  $\beta$ -82, also was found as in peptide  $\beta^A T_9$ . Its presence confirms the hydrolytic cleavage between Positions 80 and 81. No tripeptide containing aspartyl or asparaginyl along with leucine and lysine was found and none would be expected in the absence of aspartyl at Position  $\beta$ -79.

Taken together the above structural data indicate that of the two possible positions,  $\beta$ -73 and  $\beta$ -79, it is the latter position where glycyl replaces aspartyl in Hb G Hsi-Tsou.

Hb G Hsi-Tsou represents the second hemoglobin variant known to involve the  $\beta$ -79 or  $\beta$ EF3 position. The original G hemoglobin reported in 1954 by EDINGTON AND LEHMANN<sup>15</sup> was later called G Acera and shown<sup>16</sup> to be  $\beta$ 79 Asp  $\rightarrow$  Asn. Another variant found in Chinese subjects, Hb G Taichung, has the change Asp  $\rightarrow$  His at the  $\alpha$ -74 or  $\alpha$ EF3 position<sup>17</sup>.

Thus far no other subjects except the two sisters described above have been found with Hb G Hsi-Tsou. The variant apparently causes no anemia in the carrier.

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